*Case Report*

# Co-Infection with *Cryptosporidium* meleagridis and *Enterocytozoon bieneusi* in an HIV+ Colombian Patient

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**Abstract:** A 44-year-old human immunodeficiency virus-infected (HIV+) female with severe immunodeficiency Category 3 (C3) diagnosed in 2010 was admitted to hospital with acute diarrhoea. She was non-adherent to antiretroviral therapy (ART) and had a previous suspicion of respiratory symptoms with a cough that had been persisting for 15 days. Clinical examination revealed severe immune deterioration (viral load: 109,655 copies/mL; CD4+ count: 14 cells/mm3), respiratory symptoms (negative sputum Gram stain and tuberculosis culture), and neurological deterioration (serological assays negative for *Cryptococcus* spp. and *Toxoplasma gondii*). A coproculture was negative for *Campylobacter* spp., *Salmonella* spp., and *Shigella* spp. Ziehl–Neelsen staining of faecal smears revealed the presence of *Cryptosporidium* spp. oocysts. PCR testing and sequencing confirmed a concomitant infection with *C. meleagridis* and *Enterocytozoon bieneusi*. The patient was treated with metronidazole (500 mg every 8 h for 5 days) and nitazoxanide (500 mg every 12 h for 14 days). After requesting voluntary discharge and abandoning ART and parasiticidal treatments, she experienced a dramatic deterioration of her state of health and contact with her was lost. Our results have demonstrated that molecular-based testing improves the detection of opportunistic pathogens that are difficult to detect by routine microscopy, allows for transmission dynamics investigations, and assists in choosing the best chemotherapeutical option.

**Keywords:** AIDS; diarrhoea; enteric pathogens; molecular diagnosis; opportunistic infections

## 1. Introduction

The protozoan *Cryptosporidium* spp. and the microsporidian *Enterocytozoon bieneusi* are opportunistic, intracellular, enteric pathogens that are able to cause severe disease in immunocompromised patients, including those infected with human immunodeficiency virus (HIV) [1,2]. Microscopy detection of both agents in stool samples is challenging, requiring specific staining protocols and specific skills. In contrast, PCR-based methods provide improved diagnostic sensitivities, allow species/genotype identification, and allow for studies of transmission dynamics. Nitazoxanide is the only licensed drug for the treatment of cryptosporidiosis, but it has failed to demonstrate convincing effectiveness among HIV+ patients [3]. Albendazole and fumagillin are the drugs of choice for the treatment of human microsporidiosis [2]. Because the efficacy of both drugs is microsporidial species dependant, early differential diagnosis of *E. bieneusi* is important to prompt adequate chemotherapy and patient management [4]. Here, we report a co-infection with

zoonotic *C. meleagridis* and *E. bieneusi* in a severely immunocompromised HIV+ patient in Medellín, Colombia.

## 2. Case Description

A 44-year-old HIV+ female attended a medical consultation in January 2021 with a cough that had been persisting for approximately 15 days. She was afebrile, with no diarrhoea, had a severe headache, and had experienced weight loss. The patient had a history of poor adherence to antiretroviral therapy (ART, 300 mg tenofovir, 200 mg emtricitabine, 300 mg atazanavir, and 100 mg ritonavir once daily) but declared adherence at the time of admission, which was well tolerated. She also declared that she consumed alcohol occasionally but neither consuming psychoactive substances nor smoking. She was first diagnosed with HIV in 2010 during pregnancy (viral load: 453,083 copies/mL; CD4+ count: 28 cells/mm$^3$). At that time, the patient had a co-infection with *Pneumocystis jirovecii* and was therefore classified with an HIV infection at stage C3 (AIDS). She started ART at week three of gestation, achieving an undetectable viral load at the second trimester; however, she abandoned treatment soon after giving birth. She attempted to adhere to ART on several occasions afterwards but failed to comply with it repeatedly. Monitoring data on HIV infection status were available for October 2017 (viral load: 430,000 copies/mL; CD4+ count: 49 cells/mm$^3$) and February 2019 (viral load: 61,982 copies/mL; CD4+ count: 269 cells/mm$^3$). Lung function abnormalities compatible with tuberculosis were detected during a medical examination in January 2021 (Figure 1).
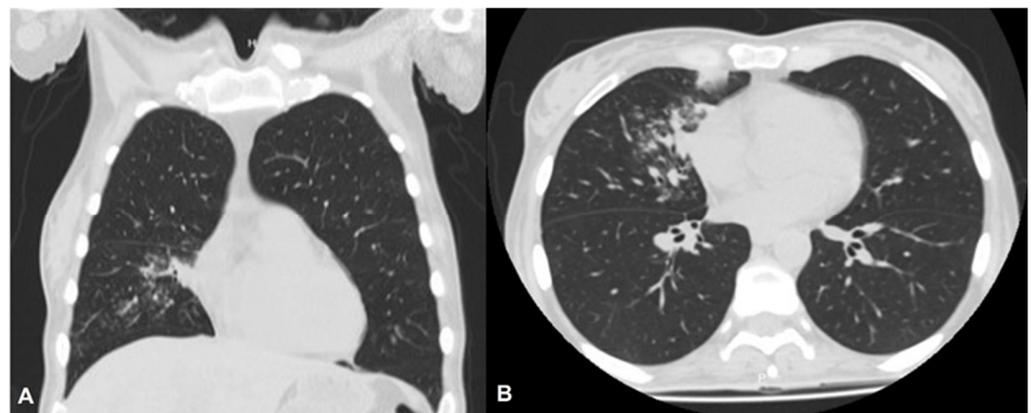


**Figure 1.** High-resolution chest tomography. (**A**) Coronal cut of high-resolution chest tomography showing expanded lung parenchyma. (**B**) Transversal cut of high-resolution chest tomography showing lung centrilobular nodules with a budding tree pattern, mainly in the middle lobe and a few in the left apical.

Chest radiography did not show signs of an increased cardiothoracic ratio or pulmonary congestion. Sputum smear microscopy was negative for alcohol-acid-resistant bacilli; the Xpert® MTB/RIF PCR assay was negative for rifampicin-resistant *Mycobacterium* complex species; and no mycobacterial growth was observed in Löwenstein–Jensen and MGIT solid media for as long as eight weeks. Antigen detection for *Histoplasma capsulatum* was negative. HIV infection status revealed a viral load of 625,664 copies/mL and a CD4+ count of 23 cells/mm$^3$, which is strongly suggestive of medication non-adherence.

The patient was re-admitted to hospital in September 2021. She presented with acute diarrhoea and severe immune deterioration (viral load: 109,655 copies/mL; CD4+ count: 14 cells/mm$^3$). The results of chest radiography demonstrated unremarkable cardiomediastinal contour with no pulmonary or pleural mass. A cytomegalovirus viral load test revealed 99 copies/mL. Neurological deterioration by monoparesis in the lower extremity was reported, but serological assays were negative for *Cryptococcus* spp. and *Toxoplasma gondii*. A coproculture was negative for bacterial pathogens including *Campylobacter* spp., *Salmonella* sp., and *Shigella* sp. Ziehl–Neelsen staining of faecal smears revealed the pres-

ence of *Cryptosporidium* spp. oocysts. The patient was treated with metronidazole (500 mg every 8 h for 5 days) and nitazoxanide (500 mg every 12 h for 14 days). Then, the patient requested voluntary discharge. A follow-up call was made at the end of November 2021. The patient's health condition was very poor. She had interrupted ART and parasiticidal treatments three weeks before and had refused to eat. Contact with the patient was lost after that.

The total DNA was extracted and purified from 200 mg of faecal material. As part of an ongoing international collaborative research project, the DNA sample was shipped to the Spanish National Centre for Microbiology (Majadahonda, Madrid) for further molecular testing. *Cryptosporidium* species and subtype family identification was carried out using PCR protocols targeting the small subunit ribosomal RNA (*ssu* rRNA) and the 60 kDa glycoprotein (*gp60*) genes of the parasite [5,6]. Additional parasite testing also revealed the presence of *E. bieneusi* using a PCR protocol targeting the internal transcribed spacer (ITS) region of the microsporidia [7]. Sanger sequence analyses of the corresponding PCR products confirmed the presence of *C. meleagridis* subtype IIIgA20G3R1 and *E. bieneusi* genotype WL11. The nucleotide sequence data obtained in this study have been deposited in GenBank under the accession numbers ON175815, ON204001 (*C. meleagridis*, *ssu* and *gp60* loci), and ON176478 (*E. bieneusi*, ITS locus). PCR-based testing was negative for other microeukaryotic species, including *Cyclospora cayetanensis*, *Cystoisospora belli*, *Giardia duodenalis*, *Entamoeba histolytica*, *Entamoeba d*ispar, and *Blastocystis* sp. [8–12].

## 3. Discussion

Severely immunocompromised HIV+ patients (CD4+ counts $\leq$200 cells/mm$^3$) are particularly vulnerable to opportunistic pathogens, such as the protozoan *Cryptosporidium* spp. and the microsporidian *E. bieneusi*. Under this circumstance, infections with these agents often lead to life-threatening, persistent diarrhoea, dramatically worsening the health status and welfare condition of patients [1,2]. In addition to fast, accurate, and sensitive pathogen detection, molecular-based methods allow for species/genotype identification. This information is relevant for (i) investigating the source of infection, including zoonotic transmission, and (ii) selecting the most effective chemotherapeutical approach for a given pathogen.

The genus *Cryptosporidium* comprises at least 44 species, of which *C. hominis* and *C. parvum* are responsible for nearly 95% of the human cryptosporidiosis cases documented globally [13]. The third most common species is bird-adapted *C. meleagridis* [13]. Our patient was infected with *C. meleagridis* subtype IIIgA20G3R1. Other members of the *gp60* subtype family IIIg have been previously identified in HIV+ patients in Thailand [14] and in poultry in Brazil [15], suggesting that both zoonotic and anthroponotic transmission pathways are possible. It is unknown whether the patient had regular contact with birds or their excreta. Of note, *C. meleagridis* IIIbA26G1R1 has been recently reported in a 1-year-old toddler attending a day-care centre in Medellín (GenBank accession MN746323) [16].

More than 600 *E. bieneusi* genotypes have been defined and grouped into 11 phylogenetic groups [17]. Genotype WL11 (formerly known as Peru5) belongs to zoonotic Group 1. It was first described in foxes in the USA [18]. Since then, WL11 has also been identified in HIV+ Peruvian patients [19], Iranian immunodeficient patients [20], and domestic cats and dogs in Colombia [21,22]. Interestingly, two of the five previous reports documenting the occurrence of *E. bieneusi* WL11 were from Colombia.

The most clinically relevant microsporidian pathogens include *E. bieneusi* and members of the genus *Encephalitozoon* (*E. intestinalis*, *E. cuniculi*, and *E. hellem*). Differential diagnosis of disseminated microsporidiosis using PCR is crucial, as treatment for the infection is genus specific [2]. Indeed, albendazole has limited efficacy against *E. bieneusi* compared with *Encephalitozoon* spp. and other microsporidian species. These agents should also be suspected in severely immunocompromised patients with CD4 counts $\leq$200 cells/mm$^3$ who present with multi-organ involvement, including fever, renal failure, conjunctivitis, sinusitis, respiratory symptoms, and central nervous system symptoms [23]. Molecular-

based analyses not only can improve the accuracy of opportunistic pathogen detection but also provide insight into the source of infection and transmission dynamics. They can assist in the choice of the most adequate chemotherapeutical option in clinical practice.

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